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Effects of ultraviolet irradiation on the acquisition and expression of tick resistance in guinea-pigs

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Summary. In guinea-pigs it has been shown that resistance to ticks is an acquired, immunologically mediated phenomenon. It has been suggested recently that epidermal Langerhans cells (LC) may play roles in the mechanisms of resistance to ticks. The ATPase-positive epidermal LC of guinea-pigs have been shown to be depleted for a period of several days following u.v. irradiations.

In this study, u.v. treatment of guinea-pigs' ears before primary tick infestations resulted in a significant reduction of acquired resistance to ticks. When u.v. treatments were applied to resistant animals prior to the challenge infestations, a marked reduction in expression of resistance to ticks was demonstrated. These results can be interpreted to imply that functional LC in the epidermis are required for both the normal acquisition and the expression of the guinea-pig's immune responses to *D. andersoni* infestations.

INTRODUCTION

It has been established that acquired resistance to ticks is an immunologically mediated phenomenon. In guinea-pigs, acquired resistance to *Dermacentor* species was first described by Trager (1939). Wikel & Allen (1976) showed that guinea-pigs acquired resistance to *D. andersoni* ticks after one infestation with 100 larvae for 5 days. Tick-resistant animals allowed very few ticks to feed normally and reach engorgement during a challenge infestation. The dry weights of the fed ticks also decreased. Tick resistance was transferrable with lymphoid cells or serum from resistant animals. (Wikel, 1982; Brown & Askenase, 1981). The acquired resistance in guinea-pigs was found to be associated with a cutaneous basophil hypersensitivity reaction with marked accumulation of basophils at tick attachment sites (Allen, 1973). Brown *et al.* (1982) demonstrated that introducing anti-basophil serum to *Amblyomma americanum*-sensitized guinea-pigs abolished their tick resistance. Hence, basophils appeared to be an essential cell type for the expression of tick resistance by sensitized animals.

Recent work, using the ATPase staining technique, has shown that changes in epidermal Langerhans cells (LC) occurred in guinea-pigs infested with *D. andersoni* larvae (Nithiuthai & Allen, 1984a). The numbers of LC decreased significantly at the sites of tick attachment on naive animals, whereas increases in numbers of LC at these sites were demonstrated in resistant animals. Also, Allen, Khalil & Wikel (1979b) showed that dendritic cells resembling LC were able to trap tick salivary gland antigens in the epidermis of resistant guinea-pigs. LC have recently been found to play an important role in immune responses of skin to many antigens and allergens (Stingl, 1980). They are

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the only epidermal cells that have functional characteristics similar to Ia-bearing macrophages, possessing Fc and C3 receptors and Ia antigens on their surface membranes. They are relatively inactive in phagocytosis but show uptake of antigens and allergens (Shelley & Juhlin, 1977). Furthermore, *in-vitro* studies showed that LC as well as macrophages, perform antigen-presenting functions in a genetically restricted manner in the elicitation of antigen specific and allogeneic T lymphocyte proliferation (Stingl, 1980). Recent studies, however, have shown that the antigen-presenting function of murine epidermal LC may be impaired following ultraviolet irradiation (UVB) either *in vitro* or *in vivo* (Aberer *et al.*, 1982; Perry & Greene, 1982). Aberer *et al.* (1982) showed that epidermal cell suspensions, irradiated *in vitro*, were incapable of initiating syngeneic and allogeneic T lymphocyte proliferation in mouse primary epidermal cell–lymphocyte interactions. In studies on the induction of trinitrophenyl (TNP) specific delayed-type hypersensitivity (DTH) reactions in mice, Perry & Greene (1982) used epidermal cells from normal and u.v.-treated mice. The epidermal cells were coupled with TNP prior to subcutaneous or intraperitoneal inoculation into recipients. It was shown that TNP-conjugated normal epidermal cells were effective in initiating DTH in the recipients, whereas TNP-conjugated u.v.-treated epidermal cells were ineffective. In other studies of contact allergic sensitivity reactions to dinitrofluorobenzene (DNFB), Toews, Bergstresser & Streilein (1980) also showed that UVB irradiation of mice resulted in a marked reduction in the numbers of ATPase-positive LC in the epidermis, and when a sensitizing dose of DNFB was applied to irradiated skin, no sensitization occurred but DNFB-specific unresponsiveness was induced.

In guinea-pigs' skin, UVC irradiation has been shown to result in depletion of ATPase-positive epidermal LC populations for several days (Nithiuthai & Allen, 1984b). In this study, the effects of such UVC irradiation on the acquisition and expression of tick resistance in guinea-pigs have been investigated.

MATERIALS AND METHODS

Animals

One hundred and twenty male albino Hartley outbred guinea-pigs, weighing 350–450 g, were supplied from Canadian Breeding Labs Ltd., Montreal. They were fed on a pellet diet and water supplemented with ascorbic acid (10–15 mg/day/animal). The animals were housed individually in metal cages in a room not exposed to sunlight.

Ultraviolet treatment

UVC irradiation of guinea-pigs' ears was performed as described by Nithiuthai & Allen (1984b). Briefly, the dorsal side of the ear was irradiated using a General Electric Model G30T8 light source. Four daily 30-min exposures were given, providing a total dose of 318 mJ/cm^2. Such treatments have been shown to deplete the ATPase-positive LC population in the epidermis without gross inflammatory changes.

Ticks and infestations

One-month-old *Dermacentor andersoni* larvae used in the experiments were laboratory reared. Adult ticks were originally collected from the field in Saskatchewan. The larvae for each experiment came from the same egg mass in order to reduce variation in their feeding behaviour. They were held in vials at 90% relative humidity over a saturated potassium chloride solution at 24°. In experiment I, a total of 60 test animals was used. Each test animal was subjected twice to tick infestations with 100 *D. andersoni* larvae, receiving a primary 5-day infestation on the right ear followed by 7 days free of ticks and a secondary 5-day infestation on the left ear (Wikel & Allen, 1976). The ventral ear skin was taped (adhesive tape, Johnson and Johnson Co. Ltd.), allowing ticks to feed only on the skin of the dorsal side of the ear. The ticks were confined to the ears within plastic capsules (Wikel & Allen, 1976). Grooming was prevented by a plastic collar placed around the guinea-pig's neck. At the end of each 5-day feeding period, all ticks were removed from the host. Larvae were examined under a dissecting microscope and classified, regardless of size, as 'unfed', 'part-fed', 'partially engorged' or 'fully engorged' according to the following criteria: unfed, no blood visible in midgut diverticula; part-fed, blood filled finger-like diverticula clearly seen individually in the body cavity; partially engorged, body cavity almost filled with engorged gut diverticula except for a small visible gap between any two diverticula; fully engorged, blood filled diverticula completely filling body cavity. These categories are illustrated in Fig. 1. The numbers of larvae in each category were recorded. The percentage of engorged larvae was calculated using the formula:

$$\frac{\text{No. of partially engorged} + \text{No. of engorged larvae}}{\text{Total No. of infesting larvae}} \times 100.$$

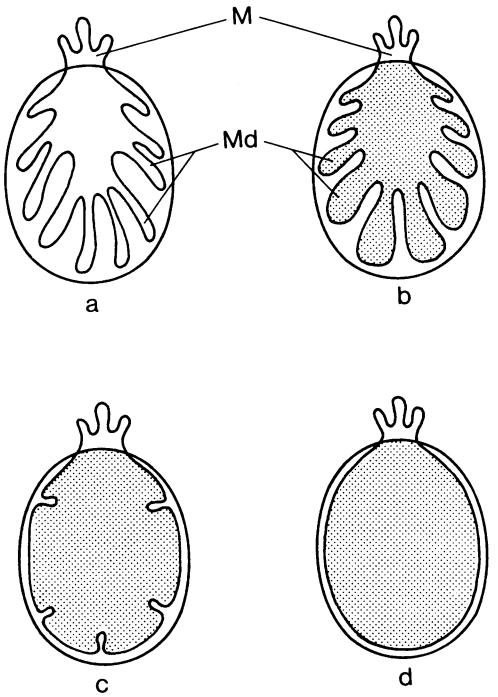

Figure 1. Diagram of various categories of *D. andersoni* larvae: (a) unfed; (b) part-fed; (c) partially engorged; (d) engorged. M, mouth part; Md, Midgut diverticula.

Larvae were then kept in 70% alcohol and the weights of larvae in each category were measured after drying them in a desiccating oven at 60° for 2 days. The dry weight of ticks from each guinea-pig was calculated using the formula:

$$\text{Mean dry weight/tick} = \frac{\text{Total weight of all larvae}}{\text{Total number of all larvae}}$$

Experimental designs

Guinea-pigs were grouped and subjected to treatments as follows.

Experiment I. This experiment was designed to investigate whether UVC irradiation of the skin prior to the primary infestation affected the acquisition of tick resistance.

Ninety guinea-pigs were divided into three equal groups, A, B and C. The dorsal side of the right ear of each animal in group A was exposed to UVC for 30 min daily on 4 consecutive days as previously described. Then the right ears of these animals and of untreated animals (group B) were subjected to 5-day primary infestations with tick larvae. After 1 week free of ticks, secondary infestations of left ears were applied to animals in both groups. In order to assess the levels of tick resistance acquired by these animals, group C animals were at the same time given primary infestations with larvae of the same batch (see Fig. 2).

Significance of differences in mean percentage engorgement and mean larval weight in different groups was assessed by Student's *t*-test.

Experiment II. This experiment was designed to investigate whether UVC irradiation of skin prior to secondary tick infestation affected the expression of tick resistance.

Thirty guinea-pigs in group A received 100 *D. andersoni* larvae in a 5-day primary infestation on the right flank. They were then maintained free from ticks

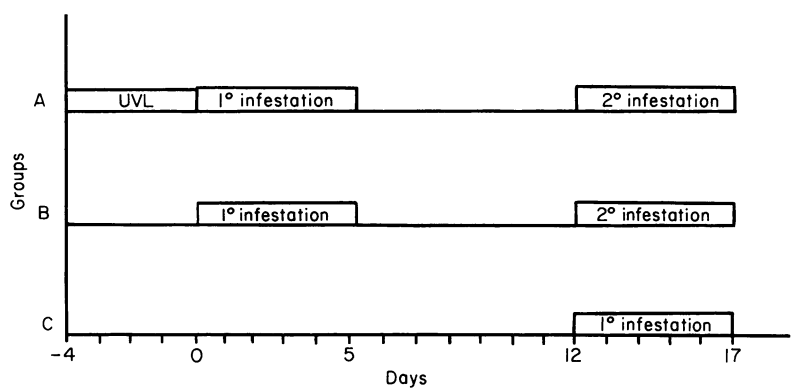

Figure 2. Diagram of experimental protocol used in Experiment 1. UVL, ultraviolet irradiation.

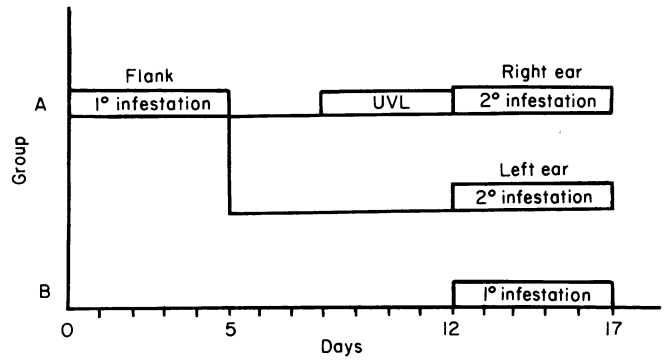

Figure 3. Diagram of experimental protocol used in Experiment II. UVL, ultraviolet irradiation.

for 1 week. On each of the last 4 days of this week, the right ears of the guinea-pigs were exposed to UVC as previously described.

Both right and left ears were then challenged with 5-day infestations of 100 larvae. At the same time, control guinea-pigs (group B) received primary infestations with larvae of the same batch (see Fig. 3).

Paired and unpaired *t*-tests were used in the analysis of data.

RESULTS

Experiment I: effects of UVC irradiation on the acquisition of tick resistance

The mean numbers of larvae which engorged during primary infestations were essentially the same on ears of irradiated and non-irradiated guinea pigs, as shown in Table 1. The mean weights of larvae were also very similar (Table 2). No statistically significant differences were evident.

When those groups of animals were subjected to secondary infestations on the opposite ear, guinea pigs that had not been irradiated (Group B) exhibited resistance to ticks. The mean percentage of larvae engorging on these animals was 28·98 as opposed to 79·21 on control (Group C) animals undergoing primary infestation with the same batch of larvae (Table 1). The mean weight of larvae was significantly less in Group B animals compared with those from Group C (Table 2).

Irradiated guinea-pigs acquired a lower level of tick resistance. The percentage engorgement of larvae in Group A was significantly greater than that in Group B (Table 1). The mean larval weights in these two groups showed the same trend (Table 2), but no statistical difference was evident.

Table 1. Mean percentage (±SEM) of *D. andersoni* larvae engorging on guinea pigs

	Groups of animals		
Tick infestations	A (u.v.-treated before 1° tick infestation)	B (Non u.v.-treated)	C (1° Control)
1°	85·58±1·59	84·60±2·13	—
	A *vs* B†		
2°	54·34±4·241	28·98±3·35	79·21±4·23
	A *vs* B**		
	2°A *vs* C†	2°B *vs* C**	

** $P<0.01$.
† No significant difference.

Table 2. Mean dry weight (±SEM)/tick ($\times 10^{-4}$ g) of *D. andersoni* larvae after 5-day infestations

Tick infestations	Groups of Animals A (u.v.-treated before 1° tick infestation)	B (Non u.v.-treated)	C (1° Control)
1°	1·44±0·05	1·35±0·06	—
	A *vs* B†		
2°	0·57±0·12	0·38±0·41	1·44±0·05
	A *vs* B†		
	A *vs* C***	B *vs* C***	

*** $P<0·001$.
† No significant difference.

Experiment II: effects of UVC irradiation on the expression of tick resistance

Significant tick resistance was exhibited on non-irradiated ears of these animals. As shown in Table 3, 32·13% larvae engorged on control ears (AC) compared to 8·7% on the ears of animals undergoing primary infestations with the same batch of larvae.

No significant tick resistance was exhibited on irradiated ears (AT) of the same animals in that 70·55% of larvae engorged on these ears (Table 3). The mean weights of larvae from both ears in the secondary infestations were significantly less than those of larvae from primary (Group B) infestations (Table 4). The mean larval weight from irradiated ears exceeded that from untreated ears, but this difference was not statistically significant.

DISCUSSION

UVC irradiation prior to primary tick infestation interfered significantly with the acquisition of tick resistance as assayed by the numbers of the larvae engorging during challenge infestation of these animals. During primary infestation, tick larvae apparently fed equally well on irradiated and non-irradiated ears (Table 1).

UVC irradiation at this dosage has been shown to

Table 3. Mean percentage engorgement of *D. andersoni* larvae (±SEM) after 5-day feeding

Groups of animals	Tick infestations	Mean % engorgement (±SEM)	
A	1°	97·08±0·81	
	2° (u.v.-treated ear)	70·55±3·83	2° AT *vs* 2° AC*
	2° (Control ear)	32·13±3·61	
B	1°	87·72±3·72	2° AT *vs* B† 2° AC *vs* B***

* $P<0·05$.
*** $P<0·001$.
† No significant difference.

Table 4. Mean dry weight (±SEM)/tick ($\times 10^{-4}$ g) of *D. andersoni* larvae after 5-day infestations

Groups of animals	Tick infestations	Mean dry weight (±SEM) per tick (1×10^{-4} g)	
A	1°	1·51±0·02	
	2° (u.v.-treated ear)	0·88±0·06	2° AT *vs* 2° AC†
	2° (Control ear)	0·73±0·04	
B	1°	1·44±0·07	2° AT *vs* B***
			2° AC *vs* B***

*** $P < 0{\cdot}001$.
† No significant difference.

cause marked depletion of ATPase-positive LC in epidermis of guinea-pigs lasting at least 6 days without causing marked gross epidermal inflammatory reactions (Nithiuthai & Allen, 1984b).

Ultraviolet irradiation (UVB) of murine skin, with a daily dose of 10 mJ/cm^2 for 4 consecutive days, has been associated with reduction in numbers of ATPase-positive epidermal LC and in contact sensitization to DNFB (Toews *et al.*, 1980). However, the ability to induce contact sensitivity returned to normal when the LC density and morphology returned to normal. Such irradiation has also been associated with alteration of antigen-presenting functions of LC (Perry & Greene, 1982). Failure to initiate DTH to TNP in mice receiving TNP-conjugated, UVB-treated epidermal cells from syngeneic donors was shown. In contrast, animals that received TNP-conjugated normal epidermal cells showed marked DTH. Sauder *et al.* (1981) undertook similar experiments and provided evidence that the hyporesponsiveness in recipients of TNP-conjugated, UVB-treated epidermal cells was due in part to the generation of suppressor T cells. These authors suggested the probability that UVB treatment may alter LC membranes in such a way that they no longer efficiently present hapten to effector or helper cells, but that they retain the potential of generating suppressor cells. In *in vitro* studies of murine primary epidermal cell-lymphocyte reactions, Aberer *et al.* (1982) showed evidence that UVB-treated mouse epidermal cells (as stimulator cells) were unable to induce syngeneic and allogeneic T lymphocyte proliferation, but that non-irradiated epidermal cells were able to do this. In guinea-pigs, Morison *et al.* (1981) indicated an effectiveness of UVB-irradiation in the reduction of contact allergic responses to DNCB.

The mouthparts of *D. andersoni* larvae, attached to guinea-pigs, penetrate just beyond the dermo-epidermal junction. It is probable that salivary secretions from the ticks are injected into the dermis and that antigenic components of the saliva could also be processed and presented by macrophages. Allen *et al.* (1979a) have also shown that tick salivary antigens are located for relatively long periods of time in the epidermis of tick-infested guinea-pigs near to attachment sites, and that these antigens may be concentrated in dendritic cells resembling LC in the epidermis. These results, together with the demonstration that the numbers of LC in the epidermis around tick attachment sites in primary infestations became markedly reduced (Nithiuthai & Allen, 1984a) are consistent with the suggestion that LC may play at least some part in the afferent arm of the immunologically mediated tick resistance response, acting as they are thought to do in the afferent arm of contact hypersensitivity reactions (Stingl, 1980; Silberberg-Sinakin, Baer & Rosenthal, 1976).

In the second experiment, guinea-pigs were sensitized by primary infestations of flank skin and then their tick resistance was assayed by secondary challenge infestations of both ears, only one of which had been irradiated. Judging by the percentages of larvae engorging on the untreated and treated ears, resistance was expressed on the former but not on the latter.

Silberberg-Sinakin, Baer & Thorbecke (1978) and Stingl (1980) have postulated that LC may play a role in the efferent arm of contact hypersensitivity reac-

tions, acting as antigen-laden target cells which, in the sensitized animals represent early foci of humoral and cell-mediated immune reactions in the epidermis.

Results from experiment II could be explained by the u.v. irradiation causing marked reduction in the numbers of functional LC in the epidermis, thereby removing the normal early foci for initiating epidermal lesions which characteristically develop as epidermal vesicles, heavily infiltrated with basophil leucocytes, in challenged resistant animals (Allen, 1973). However, it cannot be claimed that these changes in LC were the only effects produced by the u.v. irradiation. Further studies would be required to confirm this hypothesis.

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